Transduction of a *Proteus vulgaris* Strain by a *Proteus mirabilis* Bacteriophage

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SUMMARY

Only *Proteus vulgaris* strain PV127 out of many *P. vulgaris*, *P. morganii* and Providence strains was transduced to kanamycin resistance by high-frequency transducing variants, 5006MHFTk and 5006MHFTak, of phage 5006M, a general transducing phage for *P. mirabilis* strain PM5006. The phages adsorbed poorly to strain PV127 and did not form plaques. The transduction frequency of PV127 by these phages was 5×10^{-8} /p.f.u. adsorbed. Phage 5006M increased the transduction frequencies. Abortive transductants were not detected. Transductants segregated kanamycin-sensitive clones at high frequency and this, together with data from the inactivation of transducing activity of lysates by ultraviolet irradiation, indicated that transduction was by lysogenization. The general transducing property of the phages was not expressed in transductions to auxotrophs of PV127. Transductants (type I) resulting from low multiplicities of phage input adsorbed phage to the same extent as PV127. This suggested a defect in the transducing particles (or host) because single phage 5006M infection converted strain PM5006 to non-adsorption of homologous phage. Type I transductants did not liberate phage, suggesting a defective phage maturation function. Transductants (type II) which arose from higher multiplicities of phage input did not adsorb phage, indicating possible heterogeneity among transducing particles. Phage derived from type II transductants adsorbed poorly to PV127 and transduced it to kanamycin resistance at frequencies similar to those of phages 5006MHFTk and 5006MHFTak, ruling out host-controlled modification as a cause of the low transduction frequencies. This phage transduced PM5006 to antibiotic resistance at high frequencies but generalized transduction was again not detected. It was suggested that general transduction could be performed by particles which, due to a different composition and/or mode of chromosomal integration, made material they carried susceptible to host-cell modification.

INTRODUCTION

Proteus mirabilis, *P. vulgaris*, *P. morganii*, *P. rettgeri* and Providence strains may be readily distinguished by simple biochemical tests (Carpenter, 1964). They do however have properties in common and their arrangement is confused (Coetzee, 1972). With the exception of *P. morganii*, strains of the remainder have similar guanine plus cytosine molar percentages (Falkow, Ryman & Washington, 1962). Although DNA from strains of *P. vulgaris* binds 90 % of *P. mirabilis* nucleotide sequences, the latter share only about 15 % of sequences with Providence and 5 % with *P. rettgeri* (Brenner & Falkow, 1971). Members of the *Proteus* group (Coetzee, 1972) have some phage receptors in common (Coetzee, 1963*a*, *b*, *c*) and host-controlled modification systems have also been described (Coetzee & Smit, 1969, 1970). However, generalized transducing phages for each of the members of the group (see Coetzee, 1972) showed strict strain specificity, with the exception of the

Table 1. *Bacteria, plasmids and bacteriophages*

Bacteria	Properties	Reference or source
<i>Proteus mirabilis</i> PM5006	Host for phages used. Cryptically lysogenic for phage 5006M	Coetzee & Smit (1970) Krizsanovich (1973) Coetzee (1974 <i>b</i> , 1975)
PM5006 <i>str-r</i>	Spontaneous mutant resistant to 1 mg streptomycin/ml	Coetzee (1974 <i>b</i>)
PM5006 <i>arg-I</i> } PM5006 <i>trp-I</i> } PM5006 <i>lys-I</i> }	Auxotrophs of PM5006	Coetzee (1974 <i>b</i>)
PM5006 <i>str-r</i> (P- <i>lac</i>)	PM5006 <i>str-r</i> with P- <i>lac</i> introduced by conjugation. The plasmid transfers the <i>lac</i> marker to other <i>Proteus</i> and <i>Escherichia coli</i> spp. at frequencies of about 10 ⁻¹ /donor cell	Coetzee (1974 <i>a</i>) Falkow <i>et al.</i> (1964)
PM5006(P- <i>lac</i> R447b)	PM5006 with the fused plasmid P- <i>lac</i> R447b introduced by conjugation. The plasmid transfers markers <i>lac</i> and kanamycin resistance to other <i>Proteus</i> spp. and <i>E. coli</i> J62-1 at frequencies of about 10 ⁻¹ /donor cell	Coetzee (1974 <i>a</i>) Hedges <i>et al.</i> (1973) Coetzee (unpublished)
<i>P. vulgaris</i> 117 strains, including strain PV127	Typical <i>P. vulgaris</i> strains; 30 sensitive to ampicillin and kanamycin, 87 including PV127 resistant to ampicillin and sensitive to kanamycin	Carpenter (1964) Dr H. C. de Klerk Dr H. J. Koornhof
<i>P. morganii</i> 60 strains	Typical <i>P. morganii</i> strains. Resistant to ampicillin, sensitive to kanamycin	Carpenter (1964)
Providence 100 strains	44 strains of subgroup A. Resistant to ampicillin, sensitive to kanamycin 56 strains of subgroup B. 40 resistant to kanamycin, sensitive to ampicillin. 16 resistant to ampicillin, sensitive to kanamycin	Carpenter (1964) Dr H. C. de Klerk Dr H. J. Koornhof
<i>Escherichia coli</i> J62-1	F ⁻ <i>lac, pro, his, trp, nal-r</i> mutant of <i>E. coli</i> K12	Clowes & Hayes (1968)
Bacteriophages 5006MHFTk	Lysate from u.v.-induced PM5006(R394)*. High-frequency transducing for kanamycin resistance	Coetzee, Datta & Hedges (1972) Coetzee (1974 <i>b</i>)
5006MHFTak	Lysate from u.v.-induced PM5006(R394)†. High-frequency transducing for ampicillin and kanamycin resistance	Coetzee (1975)
5006M	Cryptic prophage of PM5006. Produced by lytic infection of PM5006. Generalized transducing for PM5006. Serologically identical to phages 34, 5006MHFTk and 5006MHFTak	Krizsanovich (1973) Coetzee & Sacks (1960) Coetzee (1974 <i>b</i> , 1975)
5006M·PM5006 <i>str-r</i>	Phage 5006M lysate of PM5006 <i>str-r</i>	
5006M·PM5006 <i>arg-I</i>	Phage 5006M lysate of PM5006 <i>arg-I</i>	
5006M·PM5006 <i>lys-I</i>	Phage 5006M lysate of PM5006 <i>lys-I</i>	
5006M·PM5006 <i>trp-I</i>	Phage 5006M lysate of PM5006 <i>trp-I</i>	

* Superior line indicates that the plasmid was transduced to PM5006. The T compatibility R factor R394 was isolated from a strain of *P. rettgeri* and carries markers for resistance to ampicillin and kanamycin. The R factor was transferred to PM13 by conjugation. Phage 34 lysates of the ampicillin- and kanamycin-resistant progeny were used to transduce the markers to PM5006. Some transductants only expressed the kanamycin-resistance marker, and u.v. induction of these transductants yielded high-frequency-transducing phage lysates for kanamycin resistance.

† Superior lines indicate that the plasmid was transduced to a derivative of PM5006(R394).

P. mirabilis phages 34 and 13vir (Coetzee & Smit, 1969, 1970) and Providence transducing phages (Coetzee, Smit & Prozesky, 1966) which were active on a few other strains of *P. mirabilis* and Providence respectively.

The isolation from one of the *P. mirabilis* transducing phages of variants capable of high-frequency transduction (HFT) of antibiotic resistance markers (Coetzee, 1974*b*, 1975) provided a means of selection for possible transductants from strains of *Proteus* normally not sensitive to these phages. Selection of this kind has been employed by Kondo & Mitsushashi (1966) and Goldberg, Bender & Streicher (1974) (see also Gottesman, Hicks & Gellert, 1973) to extend the host range of *Escherichia coli* phage P1 to some other genera of the family Enterobacteriaceae. Encouraged by these results it was decided to test the *P. mirabilis* HFT phages on a number of *P. vulgaris*, *P. morganii* and Providence strains.

METHODS

Bacteria, plasmids and phages. These are presented in Table 1. All strains sensitive to kanamycin were tested with both 5006MHFTk and 5006MHFTak phages for transduction to kanamycin resistance. Strains which were ampicillin-sensitive were tested for transduction to ampicillin resistance by phage 5006MHFTak. Incubation temperature was 37 °C.

General phage techniques. These were according to Adams (1956) and Coetzee (1974*b*). Freeze-dried phage 34 antiserum (Coetzee & Sacks, 1960) with a neutralization constant (*K*) of 180 min⁻¹ against phages 5006MHFTk and 5006MHFTak was used.

Media and antibiotics. These were according to Coetzee (1974*b*).

Phage adsorption. The method of Kondo & Mitsushashi (1966) was used. Phage (0.1 ml) was added to 1 ml of a culture of a streptomycin-sensitive test strain in the exponential phase of growth (about 6×10^8 /ml) at a multiplicity of input (m.i.) of plaque-forming units (p.f.u.) of about 2. At 0 min and after 20 min incubation samples were diluted in ice-cold broth and titrated on PM5006str-r using media which contained streptomycin. Some tests were done with stationary-phase organisms, and adsorption temperatures of 25 and 30 °C were also investigated.

Transduction. The Millipore membrane filter technique was used (Coetzee, 1974*b*). Possible hosts were screened at a m.i. of p.f.u. of about 10, with adsorption for 40 min. In other experiments adsorption was reduced to 15 min. This was done to try and ensure individuality of infected bacteria impinged on the membranes. The phage m.i. in these experiments varied as indicated.

Selection of antibiotic-sensitive segregants. Suitable dilutions of overnight broth cultures of transductants were plated on MacConkey agar. After overnight incubation antibiotic-sensitive clones were detected by replication on to antibiotic-containing agar. All transductant and segregant clones studied were purified by streaking on the same agar. A colony was then suspended in phage 34 antiserum and incubated for 1 h before re-streaking for single colonies.

Ultraviolet irradiation of phage lysates. Ultraviolet (u.v.) irradiation was by the method of Coetzee (1974*b*).

Selection of auxotrophic or ampicillin-sensitive mutants and phage-sensitive mutants. The *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine method of Adelberg, Mandel & Chen (1965) was used. Suitable dilutions of treated cultures were plated on MacConkey agar and incubated overnight. Ampicillin-sensitive clones were screened for by replication on to agar containing the antibiotic, while auxotrophs were detected by replication on to minimal medium. Phage was also spotted on lawns of 5000 treated colonies.

Table 2. *Transduction of P. vulgaris* PV127 and *P. mirabilis* PM5006 by phage 5006MHFTk

Transductions were done as outlined in Methods. Adsorption was for 20 min, and for kanamycin resistance, membranes were incubated on nutrient agar for 1 h before transfer to MacConkey agar containing 50 µg kanamycin/ml. For detection of prototrophs, membranes were placed on minimal medium directly after filtration.

Experiment no.	Phage	Multiplicity of input (p.f.u.)	Recipient	Transduction frequency/p.f.u. adsorbed*	
				Kanamycin resistance	Prototrophy
1	5006MHFTk	10	PV127	5×10^{-8}	—
2	5006MHFTk	0.6	PV127	2×10^{-8}	—
3	5006MHFTk	0.6	PM5006	5×10^{-2}	—
4	5006MHFTk	0.6	PM5006arg-I	—	1×10^{-8}
5	5006MHFTk + 5006M	10 20	PV127	6×10^{-7}	—
6	5006MHFTk + 5006M	0.6 20	PV127	5×10^{-7}	—
7	5006MHFTk + 5006M	10 20	PV127arg-I	5×10^{-7}	$< 5 \times 10^{-9}$
8	5006MHFTk + 5006M	10 20	PV127met-I	5×10^{-7}	$< 5 \times 10^{-9}$
9	5006MHFTk + 5006M	10 20	PV127ura-I	5×10^{-7}	$< 5 \times 10^{-9}$

* Calculated for PV127 as 20 % of m.i.

Mobilization of possible non-self-transmissible plasmids. This was done according to Datta & Hedges (1972), using the plasmid P-lac and also the fused plasmid P-lacR447b, and with *E. coli* strain J62-1 as recipient on nalidixic acid-containing media.

RESULTS

Only *Proteus vulgaris* strain PV127 yielded kanamycin-resistant transductants with the HFT phages. No other transductants (abortive or complete) were detected for the remaining 276 strains. Strain PV127 was a typical *P. vulgaris* and possessed all the features which distinguish it from *P. mirabilis* strain PM5006. When the above-mentioned strains were matched on nutrient agar a demarcation line formed between their swarms (Dienes, 1946, 1947). Strain PV127 is sensitive to 3 µg kanamycin or streptomycin/ml, but resistant to 100 µg ampicillin/ml. Repeated attempts to isolate ampicillin-sensitive mutants failed but a number of auxotrophs were obtained. The ampicillin-resistance marker could not be transferred to *E. coli* strain J62-1 directly or with the use of P-lac or P-lacR447b as possible effectors (Datta & Hedges, 1972), and this resistance was presumably chromosomally located. Although both HFT phages were used in further work on PV127, results were similar and only those employing phage 5006MHFTk will be described.

Plaque formation and adsorption of phages 5006M and 5006MHFTk to strain PV127

Neither phage formed plaques or had any visible action on PV127. Stationary-phase and exponentially growing cultures were employed. Both the overlay technique and spotting

Fig. 1

Fig. 2

Fig. 1. Effect of helper phage on transduction frequency to *P. vulgaris* PV127. ○, Phage 5006MHFTk only; ×, phage 5006MHFTk with helper. Dilutions of phage 5006MHFTk were made in a constant concentration of phage 5006M·PM5006str-r. The m.i. of this non-kanamycin resistance transducing phage was 20 p.f.u.

Fig. 2. The effect of u.v. irradiation on phage 5006MHFTk. Quantitative transduction experiments were done at a m.i. of 1 p.f.u. of unirradiated phage. A type I-S segregant of *P. vulgaris* was the recipient. ○, Plaque formation on *P. mirabilis* PM5006; ●, transducing activity.

phage dilutions on surface lawns of the organism were investigated. Results of adsorption experiments varied (for no apparent reason) but no more than 20 % of input p.f.u. were adsorbed in 20 min. More than 99 % adsorbed to PM5006 in the same time. Adsorption of phage tended to be maximal with organisms growing exponentially at 37°C. Transduction frequencies were calculated on the assumption that 20 % of input p.f.u. adsorbed to PV127. In both the above aspects the phage resembled phage P1CM, the chloramphenicol-resistance-converting variant of P1 (Kondo & Mitsuhashi, 1964, 1966). This coliphage adsorbed slowly to *Salmonella typhi* which it lysogenized steadily, but it never formed plaques on this heterologous host. Phage 5006MHFTk had no visible action on any of the 5000 clones of strain PV127 which had been treated with nitrosoguanidine.

Transduction of PV127 by phage 5006MHFTk

Expts 1 to 3 (Table 2) show that kanamycin-resistant transductants of PV127 arose at a frequency of about 5×10^{-8} /p.f.u. adsorbed, compared with 5×10^{-2} /p.f.u. adsorbed with PM5006 as recipient. Phage 5006M at a m.i. of about 20 p.f.u. had a helper effect (Expts 5 and 6, Table 2) in that the transduction frequency to PV127 was increased about tenfold. The plot (Fig. 1) of transduction frequency versus m.i. in the presence and absence of phage 5006M·PM5006str-r at a constant m.i. of p.f.u. of 20, could mean that transducing particles were defective in the sense that the transduction frequency could be increased by the presence of the latter phage, but that, because of the rapid fall of the slope between m.i. of 10 and 5 p.f.u., it was unlikely that the transducing particles complemented one another (see Chan *et al.* 1972; Coetzee, 1974b). The high m.i. of helper phage required was possibly the result of poor adsorption, as a m.o.i. of 3 was sufficient for homologous transductions (Coetzee, 1974b, 1975; see also Expts 3 and 10, Table 3). The possibility that phage 5006M was merely causing a 'multiplicity effect' (see Heip, Rolfe & Schell, 1974) could not be ruled out, but is unlikely because a host-controlled modification system does not appear operative in these particular transductions (see below). Resistance to 50 µg kanamycin/ml was complete immediately after the 20 min adsorption, but resistance to 500 µg/ml was only achieved after membranes with impinged bacteria had been incubated on non-selective media for 1 h. This is similar to the transduction of kanamycin resistance to PM5006 by this phage (Coetzee, 1974b). Phage 5006MHFTk is capable of generalized

transduction of PM5006 at low frequencies (Expt 4, Table 2) but the phage was not able to transduce auxotrophs of PV127 to phototrophy (Expts 7 to 9, Table 2).

Minute colonies possibly indicative of abortive transductants were never observed. It was thought that the 1 h pre-incubation of membranes on nutrient agar could be responsible, by allowing more time for chromosomal integration; therefore some experiments were repeated in which membranes were placed on to kanamycin-MacConkey agar directly after filtration, but minute colonies were not observed.

Properties of PV127 kanamycin-resistant transductants

Transductants retained all the distinguishing features of *P. vulgaris* and could be subdivided on the grounds of the m.i. of the transducing phage. Those produced at low m.i. (< 1) of p.f.u. (type I) did not liberate phage, either spontaneously or on u.v. induction, which could plate on PM5006. Chances of re-infection of these transductants were slight because of the low adsorption rate of the phage and the phage 34 antiserum used in critical experiments. These type I transductants presumably resulted from single-particle infection, and this would indicate that transducing particles were able to lysogenize on single infection. They were, however, defective in some maturation function, for although these transductants yielded many kanamycin-sensitive segregants, named I-S (see below), no phage active on PM5006 was liberated. These transductants still formed a Dienes demarcation line when their swarms were matched against those of PM5006, and adsorbed the phages to about the same extent as PV127. Phage 5006M or 5006MHFTk (titre 5×10^9 p.f.u./ml) when spotted on lawns of these transductants formed turbid areas surrounded by razor-thin lines of clearing. Upon 10- or 20-fold dilution of the phage this effect disappeared. Purified clones from these circumscribed areas yielded phage spontaneously and on u.v. induction. The presence of the transducing phage genome produced by single-particle infection appeared to be essential for the 'circumscribed area' phenomenon—it was not observed with PV127, I-S segregants or any other PV127 derivatives. No ready explanation for the phenomenon is available: while phage 5006M exerted a helper effect on transduction frequency (and thus presumably on lysogenization) it was unlikely (see below) that particles of phage 5006MHFTk assisted one another in this respect although another form of complementation between these particles possibly exists (see Discussion). Transductants which resulted from a phage m.i. of > 10 (type II) liberated phage spontaneously and titres of 2×10^9 p.f.u./ml on PM5006 could be obtained by u.v. induction. They failed to adsorb phages 5006M or 5006MHFTk.

A striking feature of all transductants, and in marked contrast to the stable chloramphenicol-resistant lysogens which phage PICM produced in *S. typhi* (Kondo & Mitsuhashi, 1966), was the rate at which PV127 transductants lost the kanamycin resistance. After overnight growth in broth, 5 to 15 % of colony-forming units were sensitive to $3 \mu\text{g}$ kanamycin/ml. This high segregation rate from all transductants favours the view that transduction was by addition, i.e. lysogenization (Luria, Adams & Ting, 1960; Hoppe & Roth, 1974) rather than substitution as a result of a double cross-over event. Another explanation of the high segregation rate was that the phages functioned as plasmids whose replication was not perfectly attuned to the division mechanism of PV127 (see Signer, 1969). Lack of genetic knowledge concerning the phages prevented further investigation of this aspect. One hundred and fifty-four type I-S segregants were investigated. They originated from the same number of type I transductants (15 individual experiments) which did not liberate phage. These segregants were non-lysogenic, in the sense that they too did not liberate phage, and adsorbed some phage, but with no greater efficiency than the corresponding transductants

Table 3. *Transduction by u.v.-induced lysates derived from a kanamycin-resistant P. vulgaris*
PVI27 transductant

Experiment no.	Lysate	Multiplicity of input (p.f.u.)	Recipient	Transduction frequency/p.f.u. adsorbed*	
				Kanamycin resistance	Prototrophy
1	u.v.-II	10	PVI27	4×10^{-8}	—
2	u.v.-II	0.7	PVI27	4×10^{-8}	—
3	u.v.-II	0.6	PM5006	6×10^{-2}	—
4	u.v.-II	0.6	Segregant I-S	5×10^{-8}	—
5	u.v.-II	0.6	Segregant II-Sa	$< 5 \times 10^{-9}$	—
6	u.v.-II	0.5	Segregant II-Sb	8×10^{-7}	—
7	u.v.-II	0.5	Segregant II-Sc	8×10^{-7}	—
8	u.v.-II + 5006M	0.7 20	Segregant II-Sc	6×10^{-6}	—
9	u.v.-II + 5006M	0.5 20	PVI27arg-I	5×10^{-7}	$< 5 \times 10^{-9}$
10	u.v.-II + 5006M·PM5006arg-I	0.7 3	PM5006arg-I	3×10^{-1}	$< 1 \times 10^{-9}$
11	u.v.-II + 5006M·PM5006lys-I	1 20	PM5006lys-I	1×10^{-1}	$< 1 \times 10^{-9}$
12	u.v.-II + 5006M·PM5006trp-I	5 10	PM5006trp-I	1×10^{-1}	$< 1 \times 10^{-9}$

Transductions were done as outlined in Methods and the legend to Table 2.

* Calculated for PVI27 and derivatives as 20 % of m.i.

or PVI27. Seventy-nine segregants which arose from phage-liberating transductants (type II) were also studied. These were of three types. Supernatants of overnight broth cultures of the 49 type II-Sa segregants contained phage which plated on PM5006. These segregants did not adsorb phages 5006M or 5006MHFTk. The 22 type II-Sb segregants only liberated phage on u.v. induction, while phage liberation from the eight type II-Sc segregants could not be demonstrated. Segregant types II-Sb and II-Sc adsorbed about 20 % of phage 5006MHFTk in 20 min. Neither phage 5006MHFTk nor phage 5006M was ever observed to form plaques on any segregant.

The phage adsorption experiments showed that the low kanamycin-resistance transduction frequency of PVI27 could not be explained on the grounds of host-range mutants of PVI27 capable of phage adsorption (Kondo & Mitsuhashi, 1966).

Phage 5006M·PM5006str-r could not (results not shown) transduce streptomycin resistance to PVI27, to kanamycin-resistant transductants of type I or to segregant types I-S, II-Sb and II-Sc. Small doses of u.v. irradiation to the phage lysate (Coetzee & Sacks, 1960) did not affect this.

Properties of phage derived from transductants

A type II PVI27 transductant was induced by u.v. irradiation. The lysate had a titre of 2×10^9 p.f.u./ml on PM5006, to which more than 99 % of the phage adsorbed within 10 min. The phage was neutralized at the same rate ($K = 170$) as phage 5006M by antiserum to

phage 34. It did not form plaques on PV127 type I transductants or any of the segregants, but adsorbed with similar low frequencies as phage 5006M to strains which adsorbed the latter phage. Lysates of other type II transductants had similar properties, and it was concluded that transduction of PV127 by phage 5006MHFTk was not due to a mutant of the latter phage with an increased rate of adsorption to the *P. vulgaris* strain. This lysate (and lysates of other type II transductants, not shown) could transduce PV127, segregant types I-S, II-Sb, II-Sc and PM5006 to kanamycin resistance (Expts 1 to 4, 6 and 7, Table 3). The transduction frequencies of PV127 were similar to those employing phage 5006MHFTk (compare Expts 1 and 2, Table 2) and ruled out host-controlled modification as the cause of the low transduction frequency. Kondo & Mitsuhashi (1966) also ruled out host-controlled modification in their *E. coli*-*S. typhi* P1CM transduction system on similar grounds. Using segregants of type II-Sa as recipients (Expt 5, Table 3) no transductants were obtained. The phage did not adsorb to these segregants (see above). Transduction frequencies to segregant types II-Sb and II-Sc (Expts 6 and 7, Table 3) were invariably greater than to PV127 (Expts 1 and 2, Table 3). Again no abortive transductants were observed. Provided the phage present in type II-Sb segregants had a functioning immunity system this was the exact situation where abortive transductants should have been encountered (Ozeki & Ikeda, 1968). These transductants of segregants lost the kanamycin-resistance marker at the same high rate as PV127 transductants. The segregants could be re-transduced at the same frequencies as the original segregants. Transduction frequencies were increased about tenfold by the presence of phage 5006M at a m.i. of about 20 p.f.u. (compare Expts 7 and 8, Table 3). Lysates derived from type II transductants transduced PM5006 to kanamycin resistance at the same frequencies as phage 5006MHFTk (see Expt 3, Table 2 and Expt 3, Table 3) but the general transducing properties of the latter phage (Coetzee, 1974*b*) were not evident even with the use of helper non-marker transducing phage raised on the recipient (Expts 9 to 12, Table 3). The helper phage did increase the frequency of the corresponding kanamycin-resistance transductions (compare Expts 3 and 10, Table 3). All PM5006 kanamycin-resistant transductants retained the biochemical reactions of *P. mirabilis*. Just as with phage 5006MHFTk (Coetzee, 1974*b*), transductants of PM5006 by phage derived from type II PV127 transductants at m.o.i. of <0.1 were lysogenic and failed to adsorb the phages. This could be explained by the fact that PM5006 harboured a cryptic prophage (Krizsanovich, 1973, see Coetzee, 1974*b*). Ultraviolet-induced lysates of these transductants (which also yielded kanamycin-sensitive segregants at the same high rate) yielded phage with properties similar to phage 5006MHFTk. With the use of phage 5006MHFTak to transduce PV127 to kanamycin resistance, the phage which emerged from transductants transduced PM5006 to ampicillin and kanamycin resistance although no selection for ampicillin resistance was applied to PV127 (results not shown). None of the induced phages produced abortive transductants.

Ultraviolet irradiation of transducing lysates

Plots of time of irradiation of various HFT lysates and kanamycin-resistance transduction frequencies, employing PV127 and a number of kanamycin-sensitive segregants of PV127 transductants as recipients, revealed simple exponential declines of transduction frequency as a function of time of irradiation. The example presented in Fig. 2 confirmed the conclusion reached from the high segregation rates of the kanamycin-resistance marker, that transduction was by lysogenization (either as prophage or plasmid) and not by double cross-over events between marker and the PV127 chromosome (Luria *et al.* 1960; Coetzee, 1974*b*).

DISCUSSION

Results of experiments reported here indicate that transduction by the HFT *P. mirabilis* phages was almost entirely limited to the original host. The single exception – *P. vulgaris* strain PV127 – adsorbed the phages very poorly. Even this could hardly have been detected without the use of a high-frequency antibiotic-resistance transducing phage, and some features of the system merit attention.

The method used for selection of a mutant host capable of greater phage adsorption and consequent plaque formation, was not successful (see Goldberg *et al.* 1974), and phage adsorption tests on many transductants of PV127 and segregant clones also did not yield any with a greater affinity than PV127 itself. The nature of the transduction process which occurred here was established as analogous to heterogenote formation and was to be expected in conditions where possibly too little microhomology existed for double cross-over events to occur (see Coetzee, 1972; Luria *et al.* 1960).

That transductants, formed at low multiplicities of phage input (type I transductants), adsorbed homologous phage is unique for these phages. Transductants of their host, PM5006, did not adsorb the phages. This was attributed to lysogenic conversion (Coetzee, 1961). Strain PM5006 is cryptically lysogenic for an homologous prophage (Krizsanovich, 1973) and complementation of genes of transducing particles by those of the prophage hampered assessment of possible defectiveness of the transducing phages (Coetzee, 1974*b*). Type I transductants could result from single-particle transduction by particles defective for the converting gene and defective in some maturation function, for although transductants segregated kanamycin-sensitive clones at a high rate they did not release viable phage. The fact that type II transductants did not adsorb the phages could indicate complementation of this non-adsorption function by another prophage. This could indicate a certain heterogeneity in the population of transducing particles not previously detected. However, there was no means available for testing the effect of single-particle infection by wild-type phage on PV127, and the problem remains unsolved.

Phage-adsorbing segregant types II-Sb or II-Sc, derived from non-phage-adsorbing transductants, may have arisen by permutations of the prophage excision event producing gene rearrangements which resulted in faulty phage-adsorption gene products. As phage lysates of type II PV127 kanamycin-resistant transductants transduced PV127 to kanamycin resistance at the same low frequency as phage 5006MHFT*k*, and transduced PM5006 to this resistance at the same frequency as the latter phage, host cell-modification systems did not appear to play a role in the discrepancy demonstrated. However, despite good adsorption to PM5006, the phage which originated from PV127 did not possess the general transducing properties of the parent phage 5006MHFT*k*. Strain PM5006 possesses a host-controlled modification system (Coetzee & Smit, 1970). It may well be that chromosomal material carried by the generalized transducing fraction of the HFT lysate was restricted by this system, but that material coding for kanamycin (or ampicillin) resistance escaped restriction because of its different (R plasmid) origin (see Inselburg, 1966). The host-controlled modification status of PV127 is not known, but failure to detect streptomycin-resistant transductants of PV127 by phage 5006M-PM5006*str-r*, and also the failure of the generalized transduction potential of phage 5006MHFT*k* to register, could mean that here again generalized transduction markers were restricted. However, this does not always apply because Coetzee & Smit (1969) found that generalized transducing phage 34-PM13 transduced markers of strain PM13 to a recipient, which severely restricted the phage, at a frequency one-tenth of that to strain PM13. While poor particle adsorption coupled to the

low frequency of generalized transduction (Coetzee, 1974*b*) could play a role, other factors, such as differences in composition of HFT and generalized transducing particles (Ozeki & Ikeda, 1968; see also Holloway, Krishnapillai & Stanisich, 1971) and mode of integration of the particles into the bacterial chromosome (Luria *et al.* 1960), could be important.

A surprising feature was the absence of abortive transductants in attempts at generalized, as well as kanamycin-resistance transductions. These were systems where, because of possible lack of homology, abortive transduction would be expected to occur. Demerec & Ohta (1964) found many abortive transductants in P22 transductions between *S. typhimurium* and *E. coli*, and Chakrabarty & Gunsalus (1970) encountered them in transductions between *Pseudomonas aeruginosa* and *Ps. putida*. The workers mentioned above employed general transducing phage. Failure to demonstrate generalized transduction may be accounted for by the poor adsorption and the low frequency of this process (see above). There is, however, no obvious reason why HFT defective phages that are also defective in circularization or chromosome attachment function should not produce abortive transductants (Ozeki & Ikeda, 1968). It must be assumed that transducing lysates did not contain particles of this kind.

A consistent finding was that segregant types II-Sb and II-Sc were transduced to kanamycin resistance at higher frequencies than type I-S or PVI27. Segregation rates, as well as the effect u.v. irradiation of transducing lysates had on transduction frequencies, indicated that transduction proceeded by lysogenization. It is suggested that these segregants still possessed phage genetic material which provided better (or more) phage attachment sites than type I-S or PVI27 had at their disposal. This (presumed) phage resident material, like cryptic prophage 5006M, could not substitute for the helper effect of vegetative phage, the function of which remains obscure (Coetzee, 1974*b*). However, the fact that homologous non-transducing phage exerted a helper effect in transductions to both *P. mirabilis* PM5006 and *P. vulgaris* PVI27 emphasized that the primary defect was with the phage and not its hosts.

Observations (not shown) which indicated that phages 5006MHFT*k* and 5006MHFT*a**k* behaved similarly with regard to PVI27 meant that the reactions studied were not subtle enough to detect differences or that the additional marker of phage 5006MHFT*a**k* exerted no other phenotypic effect on the phage (see Coetzee, 1975).

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